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Spatial genetic subdivision between northern Australian and southeast Asian populations of *Pristipomoides multidens*: a tropical marine reef fish species

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Abstract

Nucleotide sequence polymorphism in the left domain of the control region of the mitochondrial genome of over 700 goldband snapper (*Pristipomoides multidens*) was surveyed using both direct sequencing and innovative restriction enzyme cleavage analysis techniques. Southeast Asian populations were sampled adjacent to western Irian Jaya, northern Papua New Guinea and western Timor. Six Australian populations were sampled from adjacent to Exmouth in western Australia to Weipa in the northern Gulf of Carpentaria. The results show that significant genetic structure occurs among Indonesian and Australian waters along national boundaries; 14% of the total molecular variance among restriction site haplotypes was due to genetic distinction between Indonesian and Australian samples. Several lines of evidence suggest that gene flow does not occur freely along the northern and western Australian coastline, particularly on the northwestern Kimberley coast. Australian fisheries managers need to be alerted to the possibility of at least one genetically distinct population of this important commercial species which should be protected from over-harvesting that may otherwise lead to localised extinction and the erosion of genetic diversity. There is no convincing biological argument for the observed genetic disjunction in the Kimberley area. It may be due to the combined effect of past sea-level changes, sampling error or patterns of exploitation.

Keywords: *Pristipomoides*; Fisheries management; Northern Australia; Southeast Asia; Population subdivision; Restriction enzyme analysis; Mitochondrial DNA

1. Introduction

Most marine species have complex life histories and generally experience large amounts of gene flow (Ovenden et al., 1992; Palumbi, 1994; McMillen-Jackson et al., 1994). In marine systems, the complete

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cessation of gene flow caused by an impenetrable barrier under a vicariant scenario is probably rare due to the generally large population size and ubiquitous distribution of marine species. Rather, stochastic factors as well as natural selection, local genetic drift and behaviour influence the degree of gene flow. Gene flow may also be restricted by less stringent physical mechanisms such as environmental clines, although rarely is the nature of these well known. Flying fish on the western coast of South America are an example of species with a high potential for dispersal but inferred low gene flow (Gomes et al., 1999). The species spawns off-shore has pelagic larvae and is found in a region of intense long-shore currents; yet adjacent population samples are genetically distinct. Doherty et al. (1995) found that the magnitude of gene flow was proportional to the logarithm of the length of larval life across seven fish species in the Great Barrier Reef, Australia.

Goldband snapper (*Pristipomoides multidens*) inhabit reefs on hard bottom areas at depths of 60 to at least 180 m (Allen, 1985). It is a long-lived species with individual ages calculated from otolith growth rings of up to 30 years. The species is widely distributed throughout the tropical Indo-Pacific region from Samoa in the central Pacific to the Red Sea in the western Indian Ocean, and from southern Japan to northern Australia. *P. multidens* is the most common species caught in the deepwater trap and drop-line snapper fisheries in both western Australia and the northern territory. Post-settlement goldband snapper have been shown to be largely sedentary by an otolith composition study (Newman et al., 2000). The ratio between oxygen and carbon isotopes in goldband snapper otoliths sampled from populations along the western and northern Australian coast, and in Indonesia, was significantly different. Discontinuities in reef habitats may be an effective isolating mechanism for this species if larval dispersal is not widespread. The environmental requirements of the fertilised egg or larval stage are poorly known.

Prior to 1990, the fish communities on the northern and western Australian coast were exploited by at least four commercial fishing operations that caused measurable changes in overall species abundance and composition (Sainsbury, 1987). Japanese stern trawlers operated in the region from 1959 to 1963, Taiwanese pair trawlers from 1972 to 1987, Australian and Korean 'feasibility fishing' stern trawlers in 1979 and the fledgling Australian trap fishery since 1983. Fisheries authorities are currently formulating management plans for goldband snapper adjacent to the coasts of northern territory and western Australia. The presence or otherwise of genetically cohesive populations that straddle state boundaries is a key issue for sustainable management of the resource. The interpretation of patterns of genetic subdivision in marine species is complicated by multi-stage life histories and by the lack of knowledge about dispersal, vicariant boundaries and exploitation histories, but genetic distinctions among geographically discrete populations is irrefutable evidence for the presence of multiple fisheries stocks.

The distribution of the goldband snapper encompasses the Indo-West Pacific region, an area of extraordinary marine biodiversity (MacManus, 1985). The area is an eclectic mix of geologically recent islands and ancient continental plates that are surrounded by shallow tropical seas that have fluctuated from the present level to 150 m below the current sea level in the last 125,000 years. The Arlindo Current is thought to carry warm, near-equatorial water from the western Pacific Ocean to the Indian Ocean through the Indonesian Archipelago via the Timor Sea off northwestern Australia (Gordon and Fine, 1996). In the Australian Indo-Pacific, genetically discrete populations of goldband snapper may have been formed as a consequence of barriers to dispersal linked to changing sea levels. Alternatively, prevailing currents may have promoted dispersal from north to south across the region leading to species-wide genetic homogeneity.

The objectives of this study were to infer the extent of genetic subdivision and hence gene flow on a graded scale by testing for the presence of genetic subdivision among goldband snapper populations from Australian and southeast Asian waters. The gradations encompassed spatial gene flow among populations in northern Australian waters and on a larger scale between Australian and southeast Asian fishing areas. Genetic data were collected directly and indirectly using restriction enzymes from the control or D-loop region of the mitochondrial genome.

2. Methods

2.1. Sample collection

Adult goldband snapper between 0.35 and 0.8 m were collected from six locales (Exmouth, Pilbara,

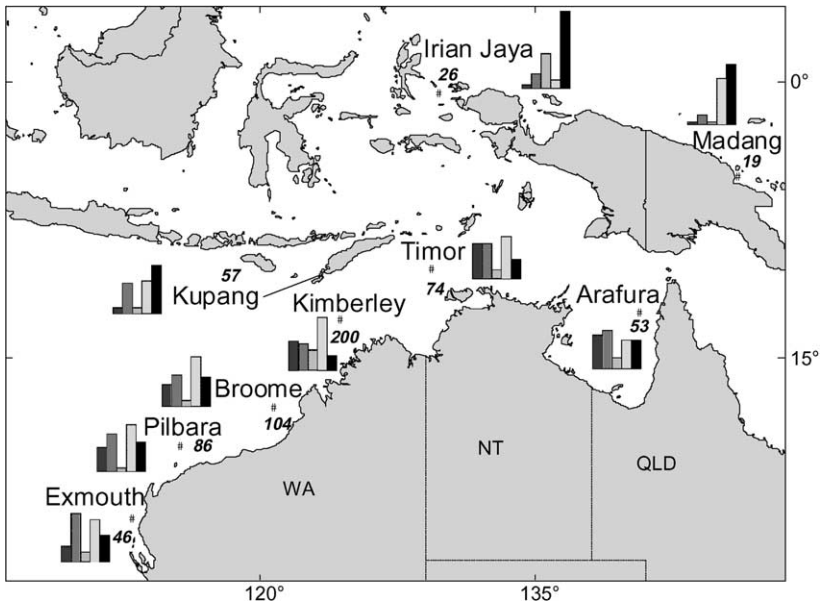

Fig. 1. Collection locales, sample size and relative frequencies of the five most common restriction site haplotypes among Australian and southeast Asian goldband snapper population. The five haplotypes were AACAA, BACAA, BACBA BACBE and BBCBA (Table 4). Australian coastline data is copyright Commonwealth of Australia, provided by AUSLIG.

Broome, Kimberley, Timor and Arafura) in Australian waters that corresponded to major areas of fishing effort (Fig. 1, Table 1). Fish for genetic analysis were sampled at random onboard research or commercial vessels as a result of either drop-line (Exmouth, Pilbara, Broome, Kimberley, Timor and Arafura) or trap fishing methods (Kimberley). Fish from three foreign locales were taken from landed catch in the Kupang fish market or opportunistically via colleagues in western Irian Jaya and Madang, Papua New Guinea.

Table 1
Number of goldband snapper analysed from six Australian localities and three southeast Asian locales with the restriction site or nucleotide sequencing technique (degrees of latitude and longitude are approximate)

Location	Latitude (S)	Longitude (E)	Restriction site	Sequencing
Australian				
Exmouth	23°6′	113°1′	46	18
Pilbara	19°7′	115°7′	86	26
Broome	17°6′	120°8′	104	–
Kimberley	13°0′	124°4′	200	19
Timor Sea	10°2′	129°8′	74	27
Arafura Sea	12°4′	140°7′	53	21
Southeast Asian				
Kupang	10°4′	123°4′	57	–
Irian Jaya	0°4′	129°8′	26	–
Madang	5°0′	146°1′	19	–
Total			665	111

Fish were sampled from Exmouth and Pilbara at the same place either on the same day or on subsequent days in 1996. At Broome, fish were collected in different years (1996, 1998 and 1999) in three different places within a 74.7 km radius. At Kimberley, fish were collected at four different places within a 121.3 km radius in 1996, 1998 and 1999. At Arafura, fish were sampled on the same day in 1996 at four locales within a 32.3 km radius. At Timor, fish were sampled on the same day in 1996 at two locales within a 29.6 km radius.

2.2. Laboratory analysis

Total DNA was extracted from muscle stored in 95% EtOH. Eight microlitres of extracted DNA template was used in a 100 μl PCR reaction with primers designed to amplify approximately 400 bp of the left domain of the control region. The primers were homologous to conserved regions in the proline gene

(5′CCACTAGCTCCCAAAGCTA 3′) and the central conserved region (5′CCTGAAGTAGGAACCAGATG 3′). This reaction mixture contained 20 mM Tris–HCl pH 8.4, 50 mM KCl, 0.2 mM of each dATP, dCTP, dGTP and dTTP, 0.5 μM of each primer, 2.5 mM $MgCl_2$, 0.1 mg/μl BSA, 4% DMSO, 3 units Taq DNA polymerase (Life Technologies) and autoclaved distilled water. Cycling conditions were: 1.5 min at 94 °C followed by 35 cycles of 5 s at 94 °C, 30 s at 50 °C and 30 s at 72 °C with a final extension of 72 °C for 5 min (Perkin Elmer 2400). The primers were also used in cycle sequencing reactions. Sequences were obtained with an ABI automated sequencer using the chain-termination method with big-dye terminators. A subset of fish from Exmouth, Pilbara, Kimberley, Timor and Arafura was sequenced. Methodological considerations precluded collecting sequence data from Broome or southeast Asian samples.

Five restriction enzymes were used for restriction site analysis of the amplicons (*Ava*II recognition site G′GA/TCC, *Alu*I AG′CT, *Dde*I C′TNAG, *Dpn*II ‘GATC, *Hin*fI G′ANTC, New England Biolabs). These enzymes were chosen because their restriction sites corresponded to polymorphic sites as determined by analysis of aligned sequence data among individuals. For restriction digests, 5–7 μl of PCR product was added to 4 μl of sterile water with 0.7 units of restriction enzyme and 1 μl of restriction enzyme buffer. Restriction fragments were visualised in 1.8–3% agarose gels (TAE buffer; 40 mM Tris, 19 mM acetic acid, 1 mM EDTA). Each sample was scored against a 25 bp DNA ladder (Gibco BRL) consisting of 18 double-stranded DNA fragments between 25 and 450 bp in multiples of 25 bp plus a fragment at 500 bp. Each new fragment pattern or morph was designated a letter name. The haplotype of each fish was a five-letter summary of morph designations. Restriction enzyme presence and absence of each of the morphs was verified by sequencing. Restriction sites were numbered according to their cleavage position, not to the position of the first base pair in the recognition sequence.

Some restriction sites overlapped, causing the presence and absence of restriction sites in the final data set to be non-independent (Fig. 2). Non-independence was removed by examining the extent of sequence variation between individuals in regions where restriction sites were known to overlap. These regions were called hypervariable regions. Examination of these regions often revealed a character that could be scored independently across all the fish. Alternatively, where sequence variation was extensive, new characters were erected and recoded to reflect as well as possible the average amount of nucleotide changes between pairs of sequences. To assist this process, alternate sequences were linked with maximum parsimony networks. The effect of other recoding schemes based on other equally parsimonious networks on the outcomes of genetic analyses has not been explored.

Fish Number	Nucleotide position						Restriction Enzyme
	1	2	3	4	**5**	6	
1	G	G	A	C	**T**	C	*Hin*fI
2	G	G	A	C	**C**	C	*Ava*II

Fig. 2. Non-independent restriction site polymorphism at six consecutive, hypothetical nucleotide positions in two fish. The sequences differ by a single site where one fish has a ‘T’ and the other fish has a ‘C’. This single substitution is responsible for the gain and loss of two different restriction sites that would lead to the incorrect calculation of two, instead of one, base pair differences between the sequences.

Lack of independence among restriction sites was removed to satisfy the assumptions of independence made by analysis techniques, especially analysis of molecular variance (AMOVA) (Excoffier et al., 1992). This method performs a hierarchical AMOVA from the matrix of squared distances between all pairs of haplotypes. Non-independence may have increased the variance of the squared distances and resulted in inflated Φ_{ST}-values that may have been less likely to be significantly different from random Φ_{ST}-values produced during subsequent permutations aimed at assessing statistical significance.

2.3. Genetic structure analysis

Genetic subdivision was assessed for goldband snapper populations using an hierarchical AMOVA implemented by the software package Arlequin v1.1 (Schneider et al., 1997). The molecular distance between haplotypes was calculated according to the

Kimura 2-parameter method (Kimura, 1980). The structure of the analysis is similar to conventional *F*-statistics, but adjusted for haploid transmission of mitochondrial genotypes (Excoffier et al., 1992). Statistical significance for the Φ-statistics is inferred from a null distribution constructed from a random allocation of haplotypes to simulated populations that have the same sample sizes as the original populations. Probability values were calculated with 16,000 permutations that are guaranteed to have less than a 1% difference with the exact probability in 99% of cases (Guo and Thomson, 1992).

Analyses were performed on molecular differentiation among restriction site and sequence-defined haplotypes under an AMOVA framework and on differences in haplotype frequencies between populations. The hypothesis of random distribution of different haplotypes between populations was performed by a modification of Fisher's exact test (Raymond and Rousset, 1995) implemented by the software package Arlequin v1.1 (Schneider et al., 1997). The probability of observing a contingency table less likely than the sample configuration under the assumption of panmixia is assessed by a random walk between the states of a Markov chain. The number of Markov chain steps was 100,000 with intervals of 3000 dememorisation steps before comparing the alternative table to the observed table. The latter type of analysis is appropriate if subdivision within the species is likely to be due only to patterns of gene flow. A combined analysis approach was adopted as it was assumed that subdivision in goldband snapper would be due to both haplotype evolution within and gene flow between populations.

To explore specific hypotheses about the genetic structure of goldband snapper populations, the significance of alternate hierarchical groupings of populations was assessed using pairwise F_{ST}'s from mutational differences among haplotypes. Haplotypes were randomly permutated 500 times between simulated populations to obtain a *p*-value that was the proportion of permutations leading to an F_{ST} larger or equal to the observed F_{ST}. Again, this method was implemented by the software package Arlequin v1.1.

2.4. Phylogenetic analysis

A matrix of pairwise mutational differences between nucleotide sequence and restriction site haplotypes was calculated using PAUP v4.0 (Swofford, 1999) and clustered using the neighbour-joining algorithm of Saitou and Nei (1987). Transversional substitutions and indels have a slower rate of evolution than transitional substitutions (Kocher and Carleton, 1997). They were weighted ten times that of transitional substitutions in the nucleotide sequence data set to reflect their relative usefulness in phylogenetic reconstruction (Nei and Kumar, 2000).

3. Results

We collected 360 bp of sequence data from the 5′ end, or left domain of the goldband snapper control region from 111 fish. Adenine was the most frequent nucleotide (36%), followed by thymine (26%) and cytosine (24%). Guanine was the less frequent (14%). Across all sequences 58 polymorphic sites were observed; all were transitions, except for four sites (numbers 185, 217, 228 and 265; Table 2) that were transversions. There were two insertion/deletion events at positions 105 and 143. The consensus sequence showed similarity with other teleost mtDNA sequence (Table 2). The first 20 bp adjoined a region coding for the tRNA proline and the 19 bp priming site used to generate the sequence that lay 10 bp beyond that. The remainder of the sequence was control region sequence. The degree of polymorphism in the left domain of the goldband snapper control region was similar to that reported by Fujii and Nishida (1997) for the Japanese flounder (*Paralichthys olivaceus*). They reported 54 sequence haplotypes from 55 fish, while this study found 61 haplotypes from 111 fish. Across the 424 bases of Japanese flounder sequence there were 126 polymorphic sites, while this study found 58 polymorphic sites across the 360 bp stretch of control region.

Amongst the 111 fish sequenced, 61 different sequence haplotypes were identified. Each haplotype has been lodged on GenBank (accession numbers AF192805-AF192865; http://www.ncbi.nlm.nih.gov/). The majority (39) of the sequence haplotypes was found only once. One haplotype was common enough to be found at all five Australian sampling locales and a further four of the 61 haplotypes were found at three to four sampling locales. The remaining haplotypes were found more than once at the same sampling

Table 2
Nucleotide sequence (380 bp) of the mitochondrial DNA of the 5′ end of the goldband snapper control region[a]

```
       ↓----TAS-----↓  ↓-Control Region--->
                        ↓-Cichlid Homology-
TTAGTTAAAC TACTTTTTGC GTAATGCATA TATGTATTAA
1                    * *
----↓
CACCATACAT TTATATTAAC CAATATCAAT ATTAGTCAAG
41       * ***   **                * *   *

GACATAACTG TTTTATCAAC ATTA:CTCGG ACCACAACAT
81     *   *               *       * **

TCACTCACCA CCATAAACCT ACAGAAATAC ATAAAGCTAA
121 * **   *       **    ****       **   *

CCCTCATTAA TCAAACAATC TAGGATCCAC AGCTGGCGAA
  ***              ** ******* *   *       *
161

ACTTAAGACC GAACACATCC GTCCACAATC CTAATATATA
201              * *         *
                                       ↓--
CCAAGGACTC AACATCCCGC CATAACTCAG AATCTTAATG
241     *                   **   *
------CCR--------↓
TAGTAAGAAC CGACCATCAG TTGATTGCTT AATGCCTACG
  *
281
TTTAATGAAG GTGAGGGACA AGACTCGTGG GGGTTTCACT
321     ** *       *     *  *
           ↓-----CSB-D-------↓
CAGTGAACTA TTCCTGGCAT CTGGTTCCTA CTTCAGGAGG
* *   **    *                          *
361
```

[a] The first 20 bp corresponds to the 3′ end of the $tRNA^{Pro}$. The location of the putative termination associated sequence (TAS, Faber and Stepien, 1997), the first 19 bp of the central conserved region (CCR, Lee et al., 1995) and the conserved sequence block (CSB-D, Lee et al., 1995) are shown. A region of 23 consecutive bases was 100% homologous with 23 bases from the 5′ end of the cichlid (*Champsochromis spilorhynchus*, Lee et al., 1995) control region (cichlid homology). Asterisks mark the 58 polymorphic base pairs. Polymorphism at position 110 was inferred from RFLP data only. Among individuals, positions 105 and 143 were represented by 'A' or an indel (:).

locale, or found at two locales. Sequence haplotype diversity that is equivalent to expected heterozygosity for diploid data varied from 0.73 to 0.89 per sampling locale. At the nucleotide level, diversity ranged from 0.019 ± 0.011 to 0.030 ± 0.016 between fish sampled from each locale.

The presence or absence of 19 polymorphic characters was scored using the RFLP technique on 667 goldband snapper from Australia and southeast Asia. The RFLP characters consisted of six regular and 13 recoded characters (Table 3). The six regular characters were the presence or absence of *Alu*I, *Dde*I and

Table 3
Character states (0—absent, 1—present) for original and recoded (bold) characters that were used to define each haplotype from the restriction site data set[a]

Character name																			
Haplotype	*Alu* 106	**Site 112**	**Site 110**	*Alu* 156	*Dde* 179	**Site A**	**Site B**	**Site C**	**Site D**	**Site E**	*Alu* 192	**Site 246**	**Site 249**	**Site G**	**Site H**	**Site I**	**Site J**	*Hinf* 342	*Dde* 359
AACAA	0	1	0	0	0	0	0	0	0	0	1	1	1	1	0	0	1	1	1
AACBA	0	1	0	0	0	0	0	1	0	0	1	1	1	1	0	0	1	1	1
AACBE	0	1	0	0	0	1	0	0	0	0	1	1	1	1	0	0	1	1	1
ABCBA	0	1	0	0	0	0	0	0	1	1	1	1	1	1	0	0	1	1	1
BAAAA	0	1	0	1	0	0	0	0	0	0	1	1	1	1	1	1	0	1	0
BAABE	0	1	0	1	0	1	0	0	0	0	1	1	1	1	1	1	0	1	0
BAABF	1	1	0	1	0	0	0	0	0	0	1	1	1	1	0	0	1	1	1
BACAA	0	1	0	1	0	0	0	0	0	0	1	1	1	1	0	0	1	1	1
BACAD	0	1	0	1	0	0	0	0	0	0	1	1	1	1	0	0	1	0	1
BACBA	0	1	0	1	0	0	0	1	0	0	1	1	1	1	0	0	1	1	1
BACBC	0	1	0	1	0	0	1	0	0	0	1	1	1	1	0	0	1	0	1
BACBD	0	1	0	1	0	0	0	1	0	0	1	1	1	1	0	0	1	0	1
BACBE	0	1	0	1	0	1	0	0	0	0	1	1	1	1	0	0	1	1	1
BADAA	0	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	1	1
BADBA	0	1	0	1	1	0	0	1	0	0	1	1	1	1	0	0	1	1	1
BBABA	0	1	0	1	0	0	0	0	1	1	1	1	1	1	1	1	0	1	0
BBABD	0	1	0	1	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0
BBABJ	0	1	0	1	0	0	0	0	1	1	1	1	1	0	1	0	0	1	0
BBCAD	0	1	0	1	0	0	0	0	1	1	1	1	1	1	0	0	1	0	1
BBCBA	0	1	0	1	0	0	0	0	1	1	1	1	1	1	0	0	1	1	1
BBCBB	0	1	0	1	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1
BBDBA	0	1	0	1	1	0	0	0	1	1	1	1	1	1	0	0	1	1	1
BCCBA	0	0	1	1	0	0	0	0	1	1	1	1	1	1	0	0	1	1	1
BCCCA	0	0	0	1	0	0	0	0	1	1	1	1	1	1	0	0	1	1	1
BDCAB	0	1	0	1	0	0	0	0	0	0	1	1	0	1	0	0	1	1	1
BECAA	0	0	1	1	0	0	0	0	0	0	1	1	1	1	0	0	1	1	1
CACAA	0	1	0	1	0	0	0	0	0	0	0	1	1	1	0	0	1	1	1
CACBA	0	1	0	1	0	0	0	1	0	0	0	1	1	1	0	0	1	1	1
CACBB	0	1	0	1	0	0	0	1	0	0	0	0	0	1	0	0	1	1	1
CACBE	0	1	0	1	0	1	0	0	0	0	0	1	1	1	0	0	1	1	1
CBCBA	0	1	0	1	0	0	0	0	1	1	0	1	1	1	0	0	1	1	1
DACAA	0	1	0	1	0	0	1	0	0	0	1	1	1	1	0	0	1	1	1
DBCBA	0	1	0	0	0	0	0	0	1	1	0	1	1	1	0	0	1	1	1
EACBE	0	1	0	1	0	1	0	0	0	0	1	1	1	1	0	0	0	1	1
FACAA	0	1	0	1	1	0	0	0	0	0	1	1	1	1	0	0	1	1	1

[a] The method of recoding characters and a description of each character is in the text. Haplotypes are a composite of morph designations for the restriction enzymes *Alu*I (AG′CT), *Ava*II (G′GA/TCC), *Dde*I (C′TNAG), *Dpn*II (‘GATC), *Hin*fI (G′ANTC) restriction enzymes.

*Hin*fI sites at positions 106, 156, 179, 192, 342 and 359. Amongst the 13 recoded characters, four characters were recoded to consist either of the presence or absence of ‘G’ at position 110, ‘C’ at position 112, ‘G’ at position 246 and ‘T’ at position 249. The remaining nine recoded characters consisted of five new sites to describe hypervariable sequence among fish at positions 183–187 (sites A, B, C, D and E), and four new sites for hypervariable sequence at positions 264–274 (sites G, H, I and J). The number of RFLP haplotypes found was 35 (Table 4). As the RFLP technique detects less sequence polymorphism than direct sequencing per individual, RFLP haplotype diversities per sampling locale were lower than for the sequence data and ranged from 0.09 to 0.37. However, RFLP diversity at the nucleotide level was comparatively

Table 4
Relative frequencies of mtDNA control region restriction site haplotypes among nine locales

Haplotype[a]	Exmouth ($N = 46$)	Pilbara ($N = 86$)	Broome ($N = 104$)	Kimberley ($N = 200$)	Timor ($N = 74$)	Arafura ($N = 53$)	Kupang ($N = 57$)	Irian Jaya ($N = 26$)	Madang ($N = 19$)
AACAA	0.087	0.151	0.135	0.180	0.230	0.226	0.018	–	–
AACBA	–	–	0.010	0.010	–	–	–	–	–
AACBE	0.022	0.012	0.010	0.010	–	0.019	–	–	–
ABCBA	0.022	–	0.019	–	–	–	0.018	–	–
BAAAA	–	–	–	–	–	–	–	–	0.053
BAABE	–	–	–	–	0.014	–	–	–	–
BAABF	–	–	–	0.005	–	–	–	–	–
BACAA	0.326	0.244	0.202	0.165	0.230	0.264	0.193	0.077	0.053
BACAD	–	–	0.039	0.020	0.027	0.038	–	–	–
BACBA	0.044	–	0.019	0.120	0.041	0.057	0.018	0.231	–
BACBC	–	–	–	–	0.014	–	–	–	–
BACBD	–	0.012	–	0.005	–	–	–	0.039	–
BACBE	0.283	0.314	0.337	0.355	0.284	0.189	0.211	0.039	0.316
BADAA	–	–	–	0.005	–	–	–	–	–
BADBA	–	–	–	0.005	–	–	–	–	–
BBABA	–	–	–	–	–	–	0.018	0.039	0.053
BBABD	–	–	0.010	–	–	–	–	–	–
BBABJ	–	0.012	–	–	–	–	–	–	–
BBCAD	–	–	0.019	0.005	–	0.019	0.018	0.039	0.053
BBCBA	0.174	0.186	0.183	0.075	0.122	0.189	0.333	0.538	0.421
BBCBB	–	–	–	–	–	–	0.018	–	–
BBDBA	–	0.012	–	–	–	–	–	–	–
BCCBA	–	–	–	–	–	–	0.018	–	–
BCCCA	–	–	–	–	0.014	–	–	–	–
BDCAB	–	–	–	–	0.014	–	0.018	–	–
BECAA	–	–	–	–	–	–	0.018	–	–
CACAA	–	0.047	–	0.015	–	–	0.018	–	–
CACBA	–	–	–	–	–	–	0.053	–	–
CACBB	–	–	–	–	–	–	–	–	0.053
CACBE	–	0.012	–	0.005	–	–	–	–	–
CBCBA	–	–	0.010	0.010	0.014	–	0.035	–	–
DACAA	–	–	–	0.005	–	–	–	–	–
DBCBA	–	–	0.010	–	–	–	–	–	–
EACBE	0.044	–	–	–	–	–	–	–	–
FACAA	–	–	–	0.005	–	–	–	–	–

[a] Haplotypes composition is described in Table 3.

higher than the sequence data, as the RFLP technique targets and surveys only nucleotides that are polymorphic. Gene diversity over RFLP characters (loci) per sampling locale varied from 0.08 ± 0.05 to 0.14 ± 0.08. Five RFLP haplotypes were found in 85% of the 667 goldband snapper samples. These haplotypes were BACBE found in 196 fish, BACAA (135), BBCBA (118), AACAA (97) and BACBA (41). The most frequent of the remaining 30 RFLP haplotypes was found in 12 fish, with 17 haplotypes being found in one fish only. RFLP data from Kimberley samples collected in three subsequent years were pooled, as an exact test of haplotype frequencies showed no significant differences. Data for Broome samples were also pooled following a non-significant exact test.

There was no noticeable relationship between phylogenetic and spatial patterns between nucleotide sequence and restriction site haplotypes, and the degree of homoplasy was high. Neighbour-joining trees for the restriction site and nucleotide sequence haplotypes contained few clades that were present in

more than 70% of 500 bootstrapped replicates (trees not shown). One clade consisting of five restriction site haplotypes (BAAAA, BAABE, BBABD, BBABA and BBABJ) was present in 70% or more of the replicates. Three clades were a regular feature of bootstrapped nucleotide sequence haplotypes trees. They were the clade containing sequence haplotypes 2, 19, 25, 29, 43, 15, 60, 44 and 57, the clade containing 11 and 20, and the clade containing 12, 35, 51, 55, and 23. The haplotypes falling within these clades were not collected at the same or close-by sampling locales.

There was evidence of significant restrictions to gene flow between goldband snapper populations in Australia and southeast Asia. When the six Australian populations were grouped and compared to a group containing the three populations from Indonesia and Papua New Guinea, 14% of the total molecular variance in the restriction site data set was between groups. The frequencies of restriction site haplotypes also supported the presence of genetic subdivision between Australian and southeast Asian goldband snapper populations (Fig. 1). For example, goldband snapper collected from Madang on the northwestern coast of Papua New Guinea had two haplotypes (BAAAA and CACBB, Table 4) that were not found elsewhere in this study. Haplotype BBCBA was particularly common in Irian Jaya, Madang and Kupang being present in 54, 42 and 33% of the fish sampled. This haplotype was less common in samples taken from Australian locales. Its frequency varied from 8 to 19% across the six locales. The probability of non-differentiation from an exact test of restriction site haplotype frequencies between Australian and southeast Asian sampling locales was less than 0.01.

The unique genetic identity of Australian compared to southeast Asian samples was also reflected in the relative magnitude of intra-population genetic variation. The average gene diversity or the probability of two randomly chosen mitochondrial RFLP haplotypes being different, of the goldband snapper sampled from the three Asian locales was higher than that of the Australian locales. The foreign average gene diversity was 0.10 ± 0.06 to 0.14 ± 0.08, whereas the Australian average gene diversity was consistently 0.08 ± 0.05. The Broome sample had a slightly higher gene diversity of 0.09 ± 0.06. The Madang sample had the highest average gene diversity in this study. These fish also had the highest variability in otolith chemical composition (Newman et al., 2000) that was explained by variability in metabolic rate due the occurrence of the fish at a large range of depths and temperatures on the northeastern coast of Papua New Guinea.

Several lines of evidence from our study indicate that gene flow of goldband snapper does not occur freely along the northern and western Australian coastline. There was evidence that fish from the Kimberley region may be genetically distinct to populations in the east and south. The frequency of at least one of the five most common restriction site haplotypes (BACBA) appears to differ between the fish sampled in the Kimberley compared to the remaining Australian locales (Fig. 1, Table 4). At the Kimberley locale, the frequency was 12%. At other Australian locales its frequency was 2–6%. Similarly, the restriction site haplotype BBCBA was present in 12–19% of fish sampled from outside the Kimberley locale, but only in 7.5% of the fish sampled from within the locale. The probability of differentiation between the Kimberley samples and the samples from Broome, approximately 400 km to the southwest on the Australian continental shelf from the Kimberley was significant (Table 5) on a pairwise exact test. When zero observations were removed from the data set by including only the five most common haplotypes that were found at all six Australian sampling locales, a similar exact test suggested that samples from Kimberley were also differentiated from Pilbara (Table 5).

A distinct genetic identity for the goldband snapper population from the Kimberley region is further supported by the small, but significant ($\Phi_{ST} = 0.0118$, p-value $= 0.007$) amount of molecular genetic variance from restriction site presence or absence between the six Australian locales. When the Kimberley samples were removed from the analysis, there was no variance between populations. The unique identity of the Kimberley population was also corroborated by pairwise F_{ST}'s calculated from both restriction site presence and absence and nucleotide sequence. Comparisons between the Kimberley samples and Exmouth, Pilbara, Arafura and Timor yielded pairwise F_{ST}'s above 0.01 for each type of data: restriction site or sequence. F_{ST}'s for comparisons with Pilbara and Timor were 0.05. In contrast, pairwise F_{ST}'s between non-Kimberley locales were mainly less than 0.01 or negative (Table 5, Fig. 3).

Table 5
Test for genetic subdivision among six Australian populations[a]

	Exmouth	Pilbara	Broome	Kimberley	Timor	Arafura
A[b]						
Exmouth	–	0.16827	0.45685	0.04641	0.17745	0.33340
Pilbara	0.28998	–	0.16587	0.00640	0.02245	0.10465
Broome	0.45396	0.80220	–	**0.00107**	0.38779	0.57913
Kimberley	0.01147	**0**	**0.00200**	–	0.26638	0.16785
Timor	0.28834	0.20655	0.33838	0.09780	–	0.77526
Arafura	0.36150	0.10670	0.13412	0.01093	0.64070	–
B[c]						
Exmouth	–	$-0.00430^{0.450}$	NA[d]	$-0.00579^{0.420}$	$0.01436^{0.240}$	$0.00262^{0.360}$
Pilbara	$-0.01211^{0.882}$	–	NA	$0.05507^{0.035}$	$-0.00634^{0.553}$	$0.00690^{0.300}$
Broome	-0.01022^{0816}	$-0.00773^{0.874}$	–	NA	NA	NA
Kimberley	$0.01381^{0.090}$	$0.02245^{0.006}$	$\mathbf{0.02267^{0.002}}$	–	$0.04867^{0.043}$	$0.03193^{0.118}$
Timor	$-0.00480^{0.573}$	$-0.00173^{0.445}$	$-0.00124^{0.430}$	$0.00633^{0.120}$	–	$-0.02485^{0.915}$
Arafura	$0.00433^{0.305}$	$0.00403^{0.251}$	$0.00214^{0.290}$	$0.02900^{0.006}$	-0.00573^{0575}	–

[a] Probability values assessed at 0.05 level that were significant after a sequential Bonferroni test (Rice, 1989) are given in bold.
[b] Probability of non-differentiation following a pairwise exact test of all restriction site haplotype frequencies (above diagonal) or the five most frequent haplotypes (AACAA, BACAA, BACBA BACBE and BBCBA; below diagonal).
[c] Pairwise genetic distance between locales (F_{ST}; restriction site data, below diagonal; nucleotide sequence data, above diagonal) and *p*-values.
[d] Some comparisons were not possible, as sequence data was not collected from the Broome locale.

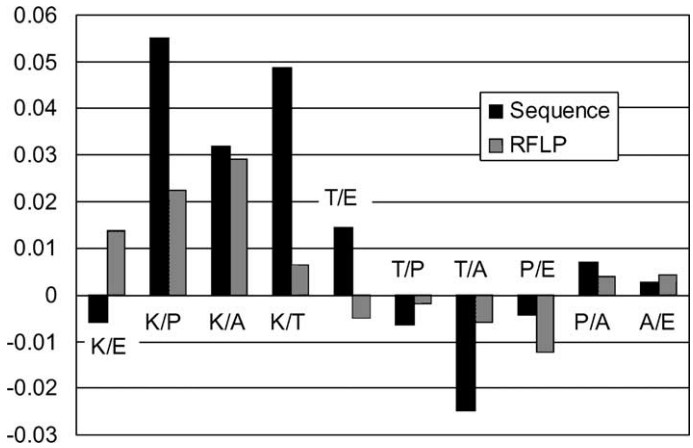

Fig. 3. Comparison of pairwise F_{ST}'s calculated from sequence and RFLP data for five populations of Australian goldband snapper (Arafura, Exmouth, Kimberley, Pilbara and Timor).

4. Discussion

Samples taken from Indonesian and Australian populations of goldband snapper were genetically distinct on either side of the Timor Sea on the northwest coast of Australia. The Arlindo Current passes from north to south and Banda Seas in Indonesia towards the northwestern coast of Australia where it contributes to the Leeuwin Current. It also contributes to the South Equatorial Current that flows from east to west (Gordon and Fine, 1996). The lack of genetic similarity between the Australian populations and the population sampled from Kupang less than 200 nautical miles away suggests that flow patterns associated

with the Arlindo Current may not facilitate gene flow across national boundaries and indeed may be partly responsible for their genetic isolation. The lack of genetic cohesiveness within Indonesian stocks of goldband snapper may also be explained by current patterns. The western Irian Jayan and northern Papua New Guinea populations of goldband snapper analysed in this paper do not lie in the pathway of the Arlindo Current. Instead they are surrounded by the Halmahera and Mindanao Eddies that transport water from west to east in coastal northern Indonesia (Gordon and Fine, 1996).

This study was unable to reject the hypothesis that there was no difference between five of the six Australian populations sampled: from Exmouth, Pilbara, Broome, Timor and Arafura. The consolidation of the five populations into a single panmictic population does not appear to make biological sense, as the five populations have disjunct distributions to the east or south of the Kimberley region. Homogeneity of goldband snapper populations distributed from Exmouth in western Australia to the Arafura Sea in northern Queensland is unlikely to be explained by extant gene flow. There are no known prevailing longshore currents in the shallow northern Australian seas that may facilitate dispersal of pelagic life stages. The distances involved are even greater than between Indonesia and Australia where gene flow has been shown by this study to be curtailed. Otolith data on northern Australian goldband snapper populations confirms that adult goldband snapper are sedentary (Newman et al., 2000), a finding that is consistent with the lack of complete panmixia reported here.

Genetic homogeneity may have occurred among populations of this presumably sedentary species as a result of distributional changes caused by past changes in sea level or by more recent fluctuations in abundance linked to past exploitation. A peak of glacial activity 125,000 years before present (ybp) is thought to have reduced northern Australian sea levels by 150 m (Chappell and Shackleton, 1986). The distribution of goldband snapper under these conditions would have been dramatically altered. Bathymetric examination shows that habitat suitable for goldband snapper would have been discontinuous consisting of a patch to the northeast of the Timor Trough and a strip from the southwest of the Trough south to the Exmouth Gulf, WA. This event was followed by a rise (115,000 ybp), a fall (18,000 ybp) and another rise (7000 ybp) in sea levels (Keenan, 1994). During these fluctuations, populations on the north and west coasts may have moved to habitat where conditions were still acceptable. These repeated moves may have continuously broken down geographical barriers permitting genetic mixing. A similar mechanism has been proposed by Coope (1979) to account for the morphological and physiological stability in the beetle fauna of Europe during the cyclical advance and retreat of glacial ice.

The genetic consequences of range alteration have been reported for other tropical species in Australian waters. Genetic analyses of saddle-tail sea perch (*Lutjanus malabaricus*, Elliott, 1996) from the northwest Australian coast and the Gulf of Carpentaria are consistent with the recolonisation after the last ice age from the western populations. Chenoweth (1999) also suggested range expansion into the shallow Arafura Sea to explain genetic patterns observed in the threadfin salmon, *Polynemus sheridani*. However, a progressive decrease in genetic diversity associated with founder events normally linked to range expansion (Keenan, 1994) has not been observed in this genetic analysis of goldband snapper.

This study shows that the goldband snapper population from the Kimberley region of northwestern Australia may be genetically distinct among Australian populations west of Torres Strait. Waples (1998) provides a framework for interpreting this kind of result. Firstly, the hypothesis could be correctly rejected but the differences between the populations are biologically insignificant. The probability of this occurring depends on statistical power, effect size, sample sizes, sample number, the number of genetic characteristics measured per fish and most importantly, the context. Secondly, the hypothesis could be incorrectly rejected due to violations of sampling assumptions. Samples taken from fisheries almost always violate the random sampling assumption; certainly the goldband snapper taken in this study were not a random sample as they were taken from amongst the commercial catch and juvenile fish were not included. Lastly, the null hypothesis could be incorrectly rejected due to chance, a type I error. However, at least one other Kimberley population of a marine species has also been found to be genetically distinct: *Penaeus monodon* (Benzie et al., 1992, 1993). More comparative studies of a wide range of taxa are needed

before the Kimberley region is given biogeographic distinction.

Assuming genetic distinction for the Kimberley stock of goldband snapper, it may have been derived from a founder event from a remote and similarly distinct population. Close-by Indonesian populations would be the most likely donors, and the Arlindo Current would be the most likely dispersal mechanism. However, this study has shown that the current is probably ineffective in mediating southward dispersal given the Kimberley stock shows no genetic affinity with any of the Indonesian populations sampled in this study at least. Furthermore, the heterozygosity of the Kimberley stock is not low as would be expected for a stock which has recently experienced a founder event (Ovenden and White, 1990). The star shape of goldband snapper restriction site haplotype phylogenetic tree is characteristic of virtually every marine species (Grant and Bowen, 1998) and is probably not the consequence of a recent bottleneck event. Rather it reflects the rapid pruning of branches that occurs when the variance of female reproductive success is high (Bermingham et al., 1998), especially in marine species (Hedgecock, 1994).

This study shows that gene flow between goldband snapper populations may be restricted on the northern and western coastline of Australia, possibly due to a disjunction in the area of the Kimberley in northwestern Australia. Fisheries managers should be alert to this and the possibility of similar occurrences elsewhere in the distribution of the species as combined with over-harvesting among reefs this could lead to localised extinctions, declining biomass and the rapid erosion of genetic diversity. Declines in genetic diversity have been linked to decreases in growth and fecundity as well as changes in sex ratio and the ability to adapt to environmental change (Chapman et al., 1999). Future research should include investigations of the larval biology of the species to test the expectation that eggs and larvae are associated with parental habitat and resist widespread dispersal.

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